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Winter Community Structure Changes in Frazil Ice and Open Water in Riverine Systems

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PURPOSE: This technical note builds on previous work (White et al. 2001, White 2002, White and Melloh 1999) that examined dissolved oxygen (DO) levels and changes in river microbiology during winter, periods of low water temperature, and periods of ice-cover, with the objective of providing guidance for winter water-quality modeling. The results of this study provide information on riverine microbial abundance and diversity in ice-affected rivers and can be applied immediately to improve winter modeling using existing water quality models such as the Corps' CE-QUAL-W2.

BACKGROUND: The U.S. Environmental Protection Agency (USEPA) issued regulations (1985, amended 1992) that implement Section 303(d) of the Federal Water Pollution Control Act known as the "Clean Water Act" (33 U.S.C. 1251-1376; Chapter 758; P.L. 845, June 30, 1948; 62 Stat. 1155 as amended). This section requires that states list waters that are impaired or threatened and for which Total Maximum Daily Loads (TMDL) must be determined. New regulations that were issued in draft form in August 1999 have not yet been implemented. By 1998, many states had listed waters that could be described as impaired or threatened. These totaled more than 300,000 miles of rivers and streams and more than 5 million acres of lakes (USEPA 2000). Both point and non-point sources of pollution, as well as natural background conditions, must be included in an analysis of TMDL to develop a mitigation plan that ensures adequate water quality under foreseeable conditions. DO is a critical component in TMDL, since adequate DO levels are necessary to sustain aquatic life and maintain acceptable water quality. DO is the fifth most frequently cited category of impairment on the 1998 303(d) lists (Table 1).

Table 1
Ten Most Frequently Cited Categories of Impairment on USEPA's 1998 303(d) Lists

Impairment	Percent of Total
Sediments	14.84
Pathogens	12.78
Nutrients	11.55
Metals	9.64
Dissolved oxygen	9.10
Other habitat alterations	5.10
Temperature	4.56
pH	4.35
Impaired biologic community	3.49
Pesticides	3.47

The concentration of DO in a river at any time is a function of the reaeration provided by tributaries, surface runoff, groundwater inflow, photosynthesis, and atmospheric absorption of oxygen (see, e.g. Tchobanoglous and Schroeder (1987)) and the deoxygenation resulting from the biochemical oxidation of organic matter in the river and sediment.

Winterbourn and Townsend (1991) report that river flow is typically dominated by groundwater inflow rather than precipitation, thus the oxygen content of groundwater sources can be an important factor in winter DO levels, particularly if runoff decreases during the winter. Freeze and Cherry (1979) note that few measurements of DO in groundwater exist, but that groundwater found in sandy or gravelly soils or where little soil overlies fractured rock can be oxygenated, while shallow groundwater in silty or clayey soils is commonly deoxygenated. Prowse (2001a) suggests that low DO levels after freezeup may occur because flow at that time consists largely of poorly oxygenated

groundwater, but that decreases in DO will be less evident where well-oxygenated surface water predominates.

The component of reaeration associated with atmospheric absorption depends on the DO saturation level (a function of water temperature and barometric pressure), the DO concentration of the water, the air-water DO concentration gradient, the area of the water surface, and hydraulic factors such as turbulence and dispersion. The maximum concentration of oxygen at the surface of the water is controlled by its saturation level, which is a function of temperature and barometric pressure. However, within the water column, the water can become supersaturated by as much as 200 percent of the saturation level (e.g., Thomann and Mueller 1987) due to photosynthetic oxygen production. The DO in a river system is used in aerobic respiration as the terminal electron acceptor during oxidation of organic and nitrogenous materials in the water column and in the oxygen demand exerted in the benthic community. The opposing processes of photosynthesis and respiration often result in a typical diurnal cycle such as that shown in Figure 1.

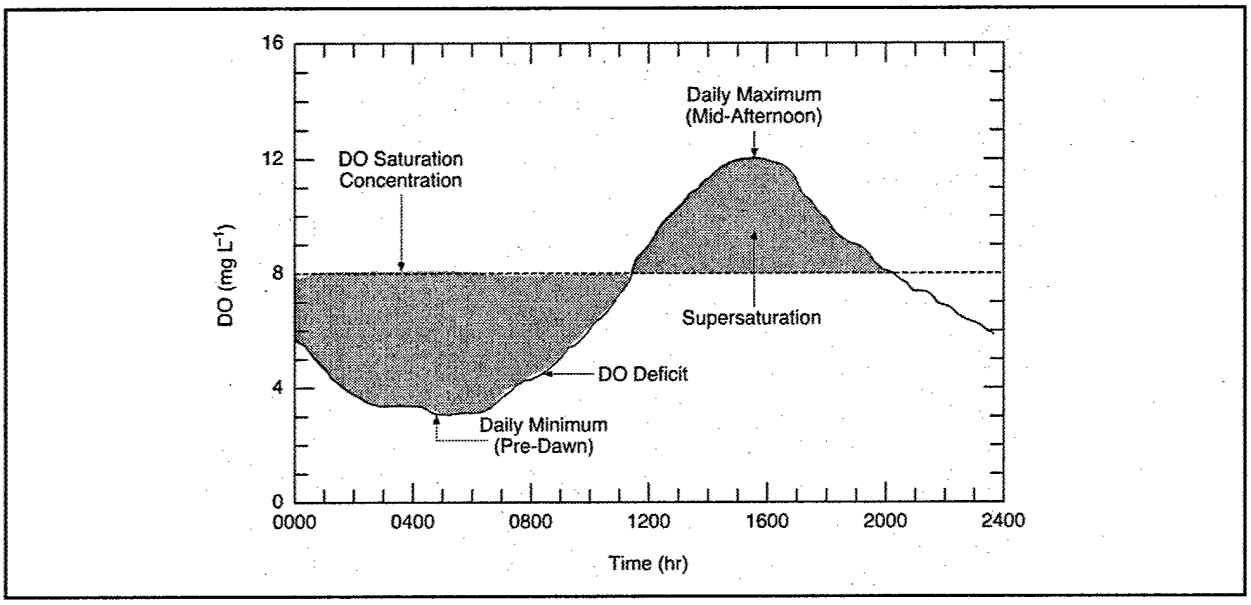

Figure 1. Typical diurnal cycling of DO in a system with photosynthesis and respiration (after Thomann and Mueller (1987))

Summer is often considered the most important period when determining TMDL because the combination of high water temperatures and low flows can result in large oxygen deficits that can decrease water quality below acceptable levels. However, previous research has documented the occurrence of wintertime oxygen deficits in ice-covered rivers. For example, DO deficits have been known to coincide with ice-cover formation (Schreier et al. 1980, Whitfield and McNaughton 1986) and can increase until ice cover breakup (Shallock and Lotspeich 1974). Rapid decreases in winter DO have been observed at river-reservoir confluences (James et al. 1992, McBean et al. 1979), with large oxygen deficits reported in deep stratified reservoirs (Gamble 1971). Despite these observations, DO processes in ice-covered rivers have received little attention, perhaps because of the logistical and economic difficulties associated with wintertime research as compared to summertime research (Prowse 2001b). In their previous study, White et al. (2001) identified several assumptions that are often made on the importance of DO in ice-covered rivers:

- Reaeration during winter is often assumed to be negligible because ice covers might provide a barrier to reaeration.
- Ice covers are also thought to prevent or minimize photosynthesis through the absorption or reflection of solar radiation in the form of visible light (e.g., Prowse et al. (2001a)).
- Because solubility of oxygen in water is higher at the lower water temperatures experienced during winter, the same magnitude of oxygen consumption due to biochemical oxidation has less effect on DO in water during the winter than during the summer.
- The microbiological processes involved in the consumption of oxygen through the decay of organic matter are thought to be insignificant during winter since they are temperature dependent, with slower rates at lower temperatures. As a result, temperature-dependent chemical rate factors have not been closely examined during winter conditions.

Building on their experiences in soil microbiology, winter water quality, and ice hydraulics, White et al. (2001) hypothesized that the effects of cyclic temperatures on biologically governed processes in streams and rivers could be greater than are currently assumed; that omission of low-temperature effects on microbial process rates may result in inaccurate predictions of water quality; and that temperature-mediated changes may influence not only rates, but may also alter the dominant microbial community, thereby causing changes in the primary metabolic pathways. Based on field and laboratory observations, combined with numerical modeling, White et al. showed that DO patterns in a small Vermont river are different during ice-covered periods than during open-water periods. In addition, diurnal cycling of DO persists during the ice-covered period, and appears to be related to photosynthetically active radiation (PAR) in a manner that suggests photosynthetic activity may be contributing to reaeration. White et al. observed algae present in the ice cover and within the frazil matrix trapped beneath the ice cover and reasoned that the porous frazil structure provides a surface for biofilm formation that supports microbial communities in a different manner than the typical riverine environment. This could explain why two independent measures found microbial communities to be different under the ice cover than in open water.

Although the presence of biofilms within river ice covers has not previously been reported, biofilm formation has been observed on a wide variety of geologic, vegetative, and other surfaces. For instance, Lock (1993) reported biofilm growth occurring in the fine sediments and gravels at depths of tens of centimeters that make up the hyphoreic zone. The presence of algal-bacterial assemblages forming biofilms within Arctic and Antarctic sea ice is well known (Ackley et al. 1979, Vincent 1988, Smith et al. 1989, Cota et al. 1991, Palmisano and Garrison 1993, Ackley and Sullivan 1994, Bowman et al. 1997). Biofilms have also been reported in snow (Weiss 1983, Thomas 1994, Hoham and Duval 2001) and freshwater lake ice covers (Felip et al. 1999). Grossman and Gleitz (1993) report close metabolic coupling of algal-bacterial communities in sea ice and in open water, with 60 percent to 90 percent of bacteria being epiphytic (attached to algae) within the ice. Field measurements by Sullivan and Palmisano (1984) revealed that about 30 percent of the bacteria were epiphytic in Antarctic sea ice and the remaining 70 percent were free-living. Smith et al. (1989) found about 37 percent epiphytic bacteria in Arctic sea ice, and estimated net bacterial production to be about 2.2 percent of net algal production in Arctic sea ice. They attributed about 40 percent of the bacterial production to epiphytic bacteria.

Several mechanisms have been suggested to explain the incorporation of the algae and other microbes into the sea ice cover (Cota et al. 1991, Palmisano and Garrison 1993). Microorganisms that collect near the bottom of the ice can become trapped by the deposition of frazil ice or thermally grown ice crystals that become buoyant and rise to the bottom of the ice cover, similar to the frazil ice deposition that occurs in rivers. Secondly, brine pockets form within sea ice as the water freezes and excludes salt, providing channels through which the microorganisms can migrate. Thirdly, microorganisms attach to the bottom surface of ice, which then undergoes thermal growth, thereby trapping the microorganisms. Finally, microbial communities can be found on the surface of the ice where the weight of snow has depressed the ice surface, allowing algae and water to flow up through cracks and flood the surface. Algal and algal-bacterial communities have also been reported in the slush layers of ice-covered freshwater lakes formed by flooding mechanisms (Felip et al. 1999). It is reasonable to infer that similar processes would result in the formation of biofilms within the ice covers of rivers. The results of a previous study (White et al. 2001) indicated that differences existed between microbial communities before, during, and after ice cover, and that more detailed study was required to understand winter microbial processes in ice-affected rivers, particularly in the presence of frazil ice deposits. This study examines changes in riverine microbial community structures based on the results of microbial sampling carried out at two intermittently ice-covered rivers in New Hampshire and Vermont.

METHODS:

Sites: Samples used in laboratory analyses of bacterial abundance and diversity were obtained from the LaPlatte River, Hinesburg, Vermont in winters 1999-2000 and 2000-2001. Because the second winter of testing on the LaPlatte River showed little frazil ice deposition, samples were also taken from the Israel River, New Hampshire in late March 2001, where thick frazil ice deposits occur nearly every winter. Location maps are given in Figures 2 and 3. Sampling data obtained during the study are summarized in Table 2. The site location, sampling methods, field and laboratory data, and analyses are described in detail in White et al. (2001) and White (2002).

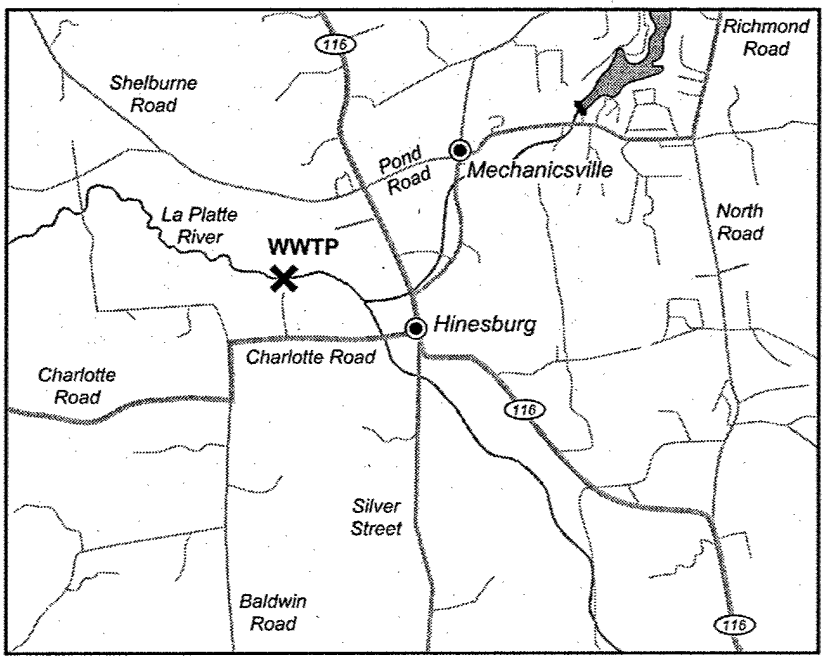

Figure 2. Location map for LaPlatte River sampling site, Hinesburg, Vermont

Approach: Microbial abundance and community structure for the LaPlatte River were measured using standard bacterial methods and were compared at different sampling dates using culturable microorganisms. The sampling times corresponded to well-established ice cover, immediately following ice breakup, and after ice out for winter 1999-2000. For winter 2000-2001, samples were collected at the LaPlatte River on six dates representing open water, intermittent ice cover, early ice cover, mid-ice-cover period, late-ice-cover period, and open water. In addition, microbial community structure within the LaPlatte River was measured during the mid-ice-cover period at two water depths: just below the ice cover and about 1 m below the ice.

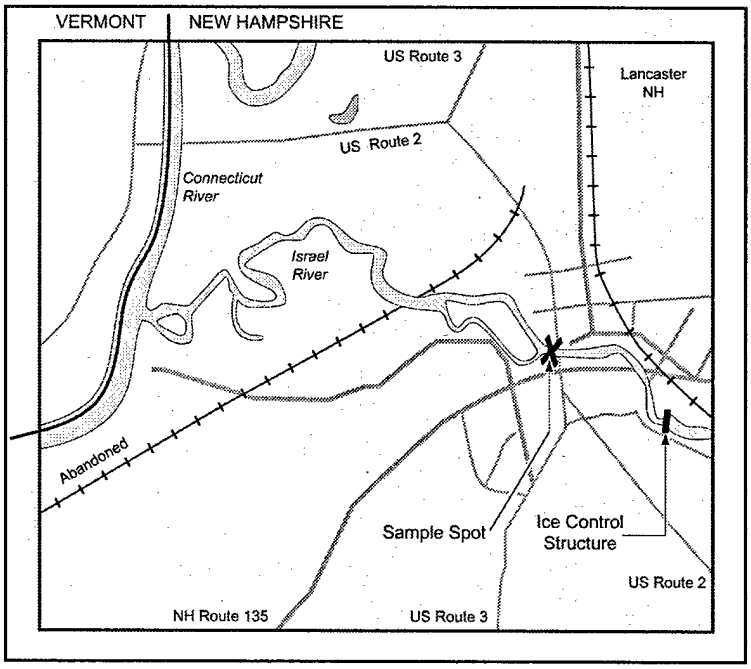

Figure 3. Location map for Israel River water and frazil sampling site, Lancaster, New Hampshire

Table 2
Monitoring and Sampling Summary

Date	Location	Type of Sample	Ice Cover
22 December 1999 through 31 March 2000	LaPlatte River	DO, pH, air and water temperature, conductivity, depth (10-min intervals)	Varied
6 January 2000	LaPlatte River	Water, microbial	Intermittent ice cover
15 January 2000	LaPlatte River	Water, microbial	Early-season ice cover
25 February 2000	LaPlatte River	Water, microbial	Late-season ice cover
29 February 2000	LaPlatte River	Water, microbial	Just after ice cover breakup
24 April 2000	LaPlatte River	Water, microbial	Open water
22 November 2000 to 7 April 2001	LaPlatte River	DO, pH, air and water temperature, conductivity (10-min intervals)	Varied
1 December 2000	LaPlatte River	Water, microbial	Open water
19 December 2000	LaPlatte River	Water, microbial	Just after ice cover breakup
3 January 2001	LaPlatte River	Water, microbial	Early-season ice cover
2 February 2001	LaPlatte River	Water, microbial (three depths)	Mid-season ice cover
23 February 2001	LaPlatte River	Water, microbial	Soon after ice cover breakup
9 March 2001	LaPlatte River	Water, microbial	Open water
29 March 2001	Israel River	Frazil (four depths), water (one depth)	Late-season ice cover
9 May 2001	LaPlatte River	Water, microbial	Open water

Sampling Schedule and Rationale. Microbial community structure within the Israel River was measured on 29 March 2001 at four depths within the frazil ice accumulation at four depths below the top of the solid ice: 30 (water surface), 91, 195, and 292 cm (Figure 4). Two water samples were obtained at a depth of 193 cm below the top of the ice (163 cm below the water surface level). These samples represent conditions in a late winter ice accumulation during a winter marked by significant frazil ice accumulation. The frazil accumulation in the Israel River was substantially formed by 27 December 2000, so that by the time the frazil and water samples were obtained at the end of March, mature microbial community development had probably occurred. Water DO, pH, temperature, conductivity, depth, and air temperatures were measured at the LaPlatte River throughout the winter. Water and ice samples were taken periodically for microbial characterization. A monitoring and sampling summary is provided in Table 2.

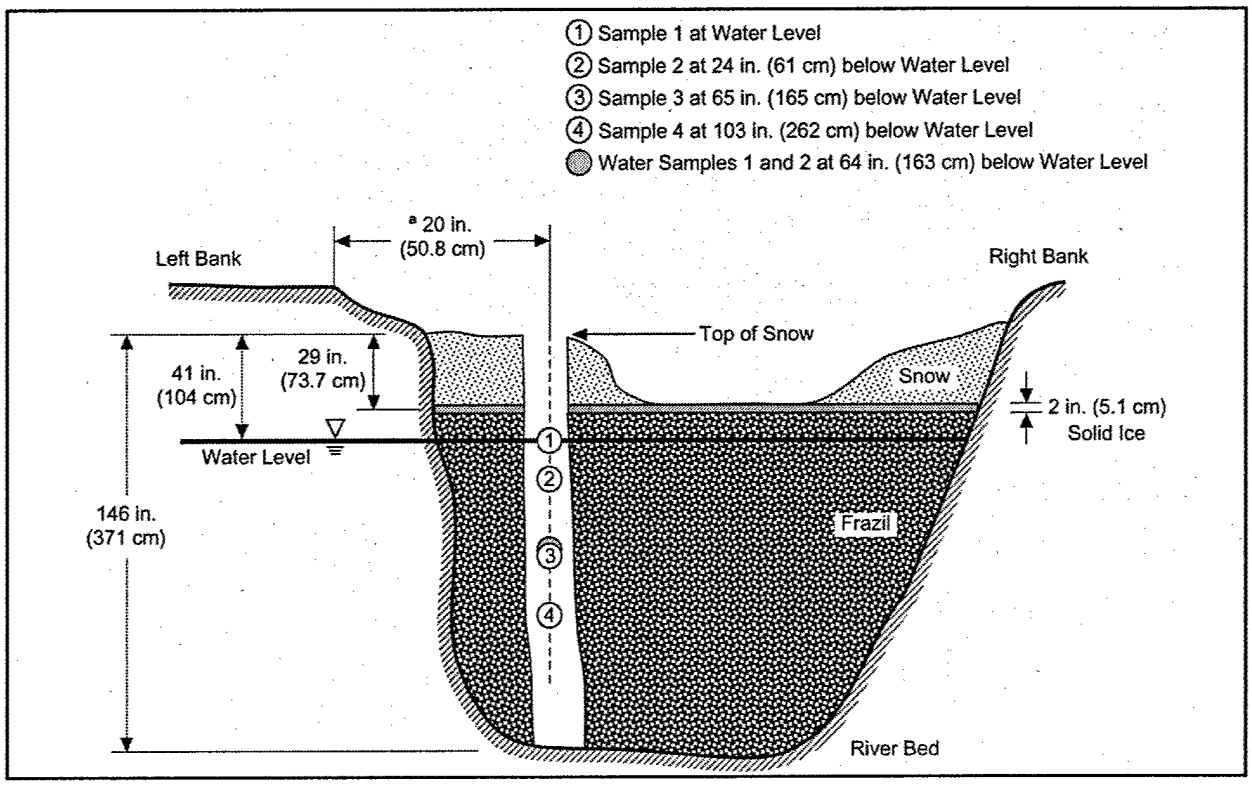

Figure 4. Profile of Israel River ice accumulation during sampling, 29 March 2001

Microbial Analyses and Characterization. Microbial community abundance and structure in river water and frazil ice samples were characterized in the laboratory. Microbial activity was measured using CRREL's temperature-controlled respirometer. Respiration measurements of evolved carbon dioxide (CO_2) provide an estimate of general, non-photosynthetic, microbial activity. The respirometer was modified to allow for simultaneous monitoring of samples incubating at different temperature regimes. Each temperature regime can be cycled in a defined amplitude and frequency schedule or held constant. Samples were incubated under low-light conditions and monitored in near-real time via headspace sampling every 4 hr. Results from the constant temperature incubations were evaluated in terms of providing initial estimates of differences for fall, winter, and spring processes for the river.

To estimate the general heterotrophic activity, respiration tests were conducted on water samples collected on three dates, 1 December 2000 (open water), 19 December 2000 (intermittent ice cover) and 2 February 2001 (mid-ice cover period), from the LaPlatte River and a single date, 29 March 2001 (late ice cover period), from the Israel River. CO_2 evolution was monitored at 0.5 °C and 10 °C. Appropriate sterile controls were included so that fluctuations caused by temperature-dependent CO_2 solubility in water could be accounted for. In addition, microbial community abundance and structure were quantified by standard bacterial methods, including plate counts for estimating bacterial abundance and a modified fatty acid procedure for characterizing bacterial communities.

The six dates of LaPlatte River sampling for microbial characterization represented late fall-early winter open water, intermittent ice cover, and mid-ice cover (Table 2) as well as early ice cover (3 January 2001), late ice cover (2 February 2001), and open water (9 May 2001). Microbial community structure within the LaPlatte River was measured on 2 February 2001 at three depths. For the LaPlatte River, the sample times represent seasonal variations from fall through winter and into early spring.

Microbial community structure within the Israel River was measured at four depths on 29 March 2001, representing the late ice cover period, during significant frazil ice accumulation.

Microbial community structure and diversity were characterized for the LaPlatte River and Israel River water samples using a modification of the fatty acid methyl ester (FAME) procedure described by Onderdonk and Sasser (1995) and MIDI, Inc. (2001). The standard procedure involves the harvesting of cells in sufficient amounts to provide measurable fatty acids (Sasser and Wichman 1991), whereas in the modified approach, the entire lawn of microorganisms is treated as a sample, rather than individual isolates. The resulting fatty acid profile is compared to a library for identification purposes. This approach provides an indication of bacterial community structure changes and can be used for describing and comparing the community structures of biofilms. Principal component analysis (PCA) was applied to the results of the modified FAME procedure using the method contained within the library (MIDI, Inc. 2001). PCA is a statistical method that can be used to empirically summarize the correlations between the variables and provide information on differences and similarities among groups.

RESULTS AND DISCUSSION: Water quality data were collected at 10-minute intervals at the LaPlatte River site between 22 December 1999 and 31 March 2000 (see Table 2) and 22 November 2000 and 7 April 2001. During the first winter, intermittent ice covers occurred between 24 and 26 December, and on 28 December, 29 December, 31 December, and 6 January. A permanent seasonal ice cover began on about 12 January 2000. By 15 January, the ice cover was 10 cm in thickness, and substantial amounts of frazil ice were deposited beneath the solid ice. Algae were visible both within the frazil ice and in accumulations at the surface in open-water areas. This ice cover remained in place, but deteriorated after mid-February with increasing sun hours, sun angle, increasing air and water temperatures, and rising water levels. Between 27 and 28 February 2000, the ice cover broke up following a snowmelt and rainfall event. Open-water areas were observed on 29 February 2000.

During the winter 2000-2001 conditions, an ice cover formed between 6 and 17 December 2000. The ice cover broke up during a sudden increase in discharge resulting from a rainfall event on 16-17 December 2000. Significant overbank flow occurred during this event. Following a period of cold weather, the ice cover re-formed again on about 24 December 2000. This ice cover remained in place until about 14 March 2001. Due to computer modem malfunction, water quality data are unavailable for the period 13 to 23 February 2001, in the middle of the ice-covered period. Ice thickness was 18 cm on 3 January 2001, including 6 cm of snow ice. Flooded and refrozen snow ice was also observed at the time of sampling on 2 February 2001. Unlike the previous winter, there were few periods of intense cold. As a result, little frazil was observed near the sampling site.

Microbial Activity. Cumulative evolved CO_2 respiration data were obtained from the CRREL respirometer using LaPlatte River water samples collected on 6 and 15 January 2000 representing intermittent and permanent ice-covered periods, respectively; on 1 December 2000 during early ice-cover formation; on 19 December 2000 in open-water immediately following a breakup event; and on 2 February 2001, an ice-covered period. The samples were evaluated concurrently at 1 °C and 25 °C using constant temperature baths for the winter 1999-2000 samples and 0.5 °C and 10 °C—to provide more realistic water temperatures—for the winter 2000-2001 samples. The results, given in Figures 5 and 6, show similar respiration patterns during the two winters. Difficulties with the respirometer prevented incubation longer than 60 hr for Israel River samples, and the results, therefore, are less definitive for the Israel River than for the LaPlatte River, because the bacterial communities were still in the lag phase of growth. However, the preliminary results indicate that respiration data of this type may be useful in characterizing winter microbial activity in rivers.

The two samples taken during winter 1999-2000 (Figure 5) both exhibited a lag period of approximately 100 hr, followed by an asymptotic increase in respiration between 100 hr and 270 hr. Both samples also behaved similarly with respect to CO_2 evolution, each evolving a cumulative total of approximately 50 µl CO_2/mL during the 270-hr incubation at 1 °C. However, the samples responded differently when incubated at 25 °C. There was significantly more CO_2 evolution from the water sample collected on 15 January 2000 under the permanent ice cover, approximately 330µl CO_2/mL, or nearly double the approximately 170 µl CO_2/mL released from the sample collected on 6 January 2000 in intermittent ice cover conditions.

The larger release of CO_2 observed in the ice-covered sample is an indication that greater bioavailable carbon, whether algal, bacterial, abiotic, or combinations of the three, is present in the ice-covered sample relative to the intermittently ice-covered sample. Although not conclusive, these data suggest that different microbial communities are present in the river during periods of intermittent ice cover compared to periods of permanent ice cover. Visual observations on 15 January 2000 revealed algae both within the frazil ice and in accumulations at the surface of open-water areas. The frazil ice matrix may have provided a favorable structure for biofilm attachment and growth. This could affect community structure in such a way that the additional algae or bacteria within the frazil matrix would increase the supply of carbon to the system relative to the open-water case, as was observed by greater release of CO_2. Or, the frazil ice may have trapped and accumulated other carbon in the river.

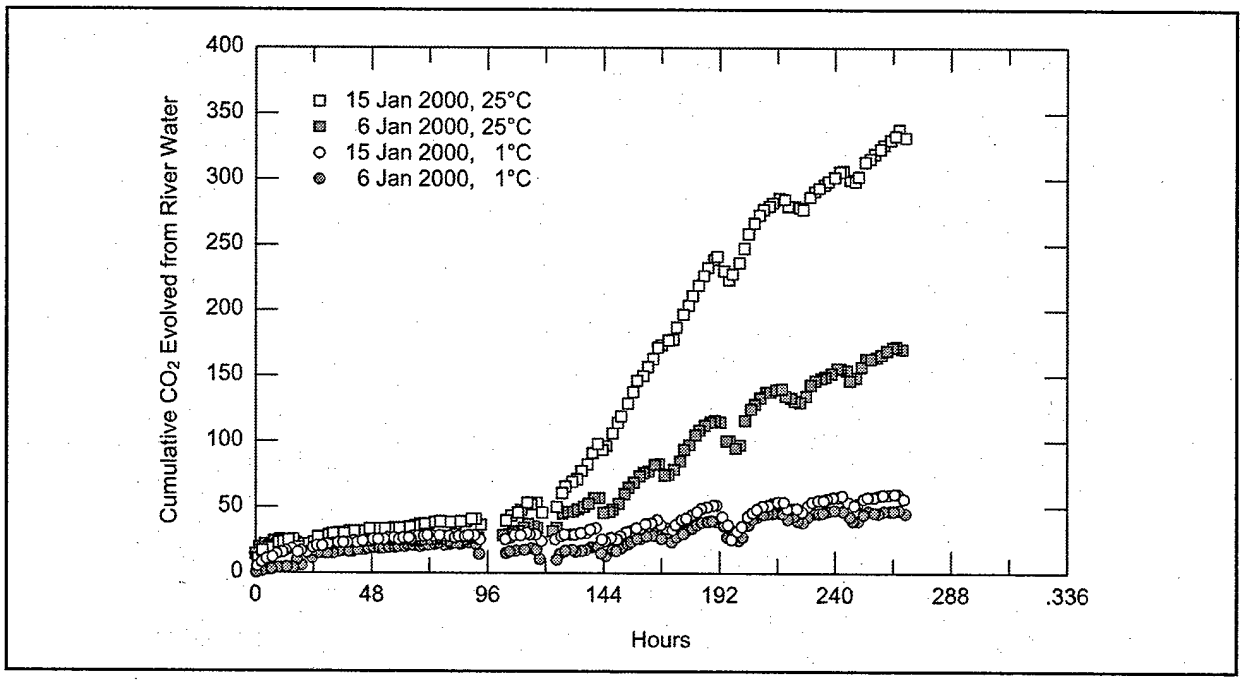

Figure 5. Cumulative respiration data for LaPlatte River water samples obtained on 6 and 15 January 2000, intermittent and permanent ice-covered periods, respectively

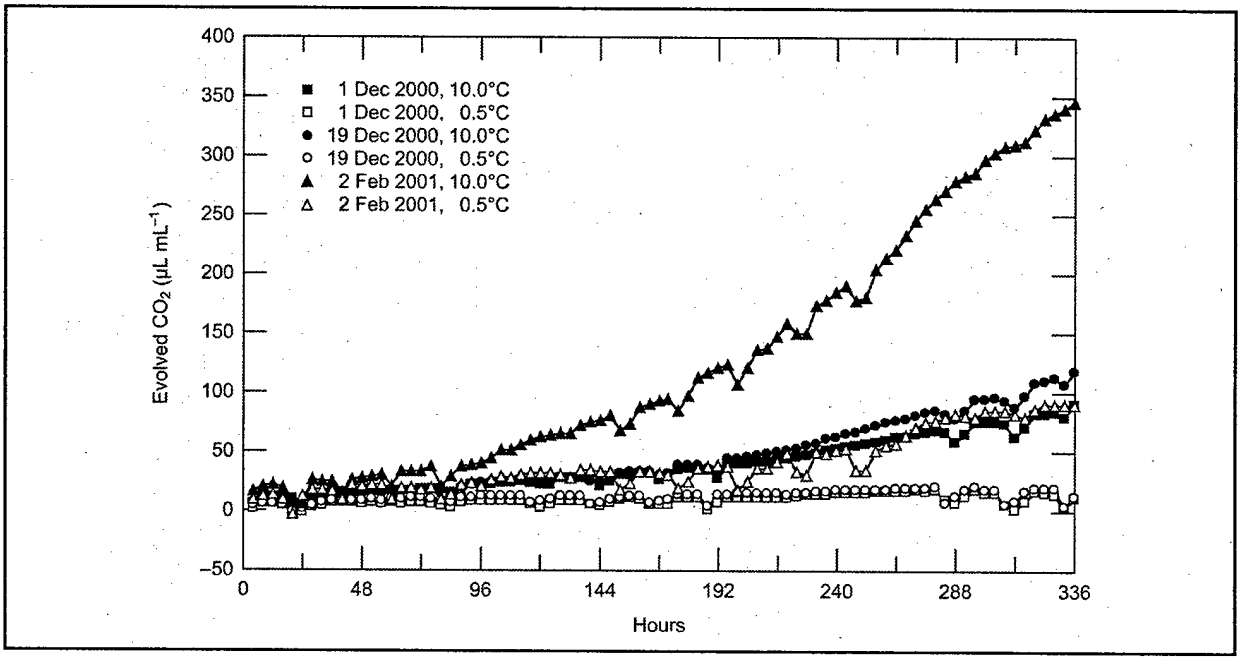

Figure 6. Cumulative respiration data for LaPlatte River water samples obtained during winter 2000-2001 for early ice formation (1 December 2000), open water following breakup event (19 December 2000), and ice-covered (2 February 2001) conditions

Microbial Abundance. The abundance of microorganisms cultured from LaPlatte River water samples obtained over a four-month period between 1 December 2000 and 9 May 2001 was quantified as shown in Figure 7. The highest recorded abundance (1.9×10^5 colony-forming units (CFU) mL^{-1}) occurred on 19 December 2000 following the high discharge and dynamic ice-cover breakup event on 17 December 2000, suggesting substantial input of microorganisms into the river system, possibly from both release of ice-associated and sediment-associated communities.

Excluding the 19 December 2000 data, microbial biomass *under* ice cover showed a general decline in cell numbers from the beginning of December 2000 ($\approx 4 \times 10^4$ CFU mL^{-1}), coinciding with lowered water temperature and intermittent ice cover formation, through the end of February 2001 ($\approx 1.5 \times 10^4$ CFU mL^{-1}). This decline in cell numbers corresponds with a decrease in the amplitude and regularity of the DO diurnal cycle as the ice cover season progressed. Following ice break-up, microbial biomass increased from $\approx 1.5 \times 10^4$ CFU mL^{-1} on 23 Feb 2001 to $\approx 3.7 \times 10^4$ CFU mL^{-1} on 9 May 2001. There was no significant difference between the mean values recorded on 1 December 2000 and 9 May 2001. These data show that microbial numbers declined in the water under an ice cover, and then, following ice breakup, rebounded to a level similar to that before the ice cover occurred (Figure 7).

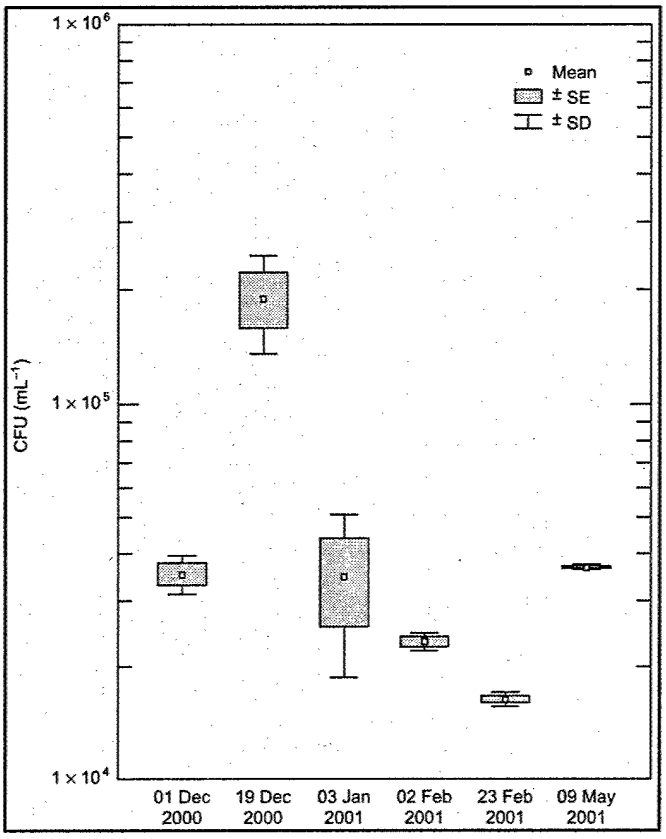

Figure 7. Plot of microbial colony forming units (CFU), representing microbial biomass in LaPlatte River water samples during winter 2000-2001

The abundance of culturable micro-organisms found in LaPlatte River water is comparable to that reported by Gordon (1970) for Alaskan rivers (10^4 mL^{-1}), but slightly lower than that reported by Felip et al. (1999) for microbial abundance in a freshwater lake ice cover (0.5×10^5 mL^{-1} to 1.5×10^6 mL^{-1}). Geesey et al. (1978) measured levels comparable to the LaPlatte River results in the water column of three snow- and ice-covered rivers (3×10^4 mL^{-1} to 5×10^4 mL^{-1}).

At the Israel River site, differences were observed in microbial abundance, both among depths in the frazil ice and between the water column and the frazil ice. Bacterial abundance in the frazil ice was an order of magnitude higher than in the water column ($\approx 10^4$ CFU mL^{-1} vs. $\approx 10^3$ CFU mL^{-1}). These results (Figure 8) are consistent with the results of Felip et al. (1999), who found higher productivity in the ice cover of a freshwater lake than in the water column below. Geesey et al. (1978) found similar results when comparing bacterial abundance in the water column ($\approx 3 \times 10^4$ mL^{-1} to 5×10^4 mL^{-1}) to that of epilithic biofilms ($\approx 5 \times 10^6$ mL^{-1} to 5×10^7 mL^{-1}).

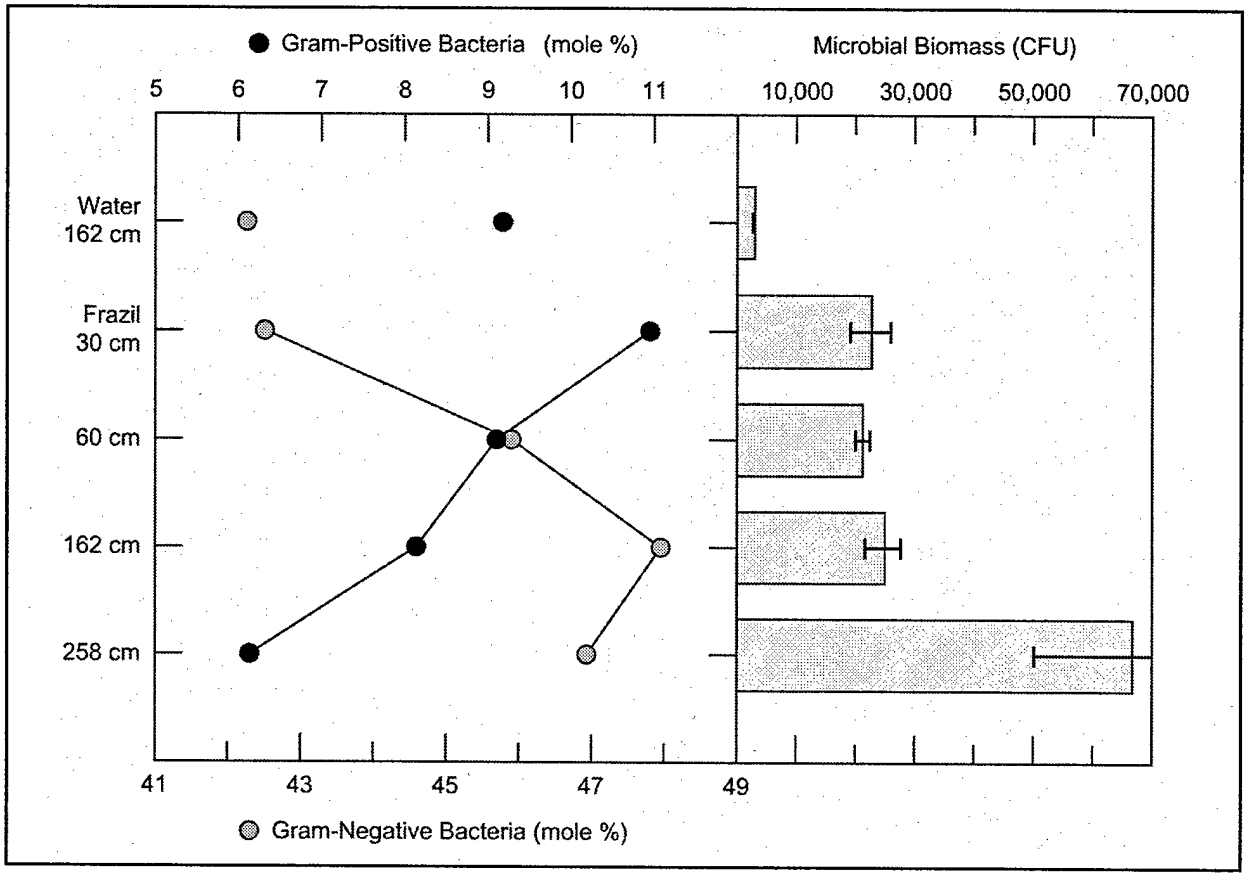

Figure 8. Relative distribution of gram-positive and gram-negative bacteria and the total microbial biomass present in water and frazil ice at varying depths in the Israel River, Lancaster, New Hampshire on 29 March 2001

Bacterial abundance was observed to increase with increasing depth in the frazil deposit, from ≈ 2.5 X 10^4 CFU mL^{-1} at the 30-cm depth to ≈ 6.7 X 10^4 CFU mL^{-1} at 258 cm. The changes in abundance with depth could be a result of structural differences within the frazil deposit, or due to the age of the deposit. A thick frazil deposit such as the Israel River deposit would be expected to be less porous near the surface than at depth, because the buoyant forces of the frazil deposited beneath will tend to compress the upper layers of the deposit (e.g., Beltaos and Dean 1981). Also, frazil deposited earlier in the season may have experienced smoothing during relatively warmer periods or freezing of interstitial areas, resulting in layers that are less permeable and less porous.

The water samples showed a greater relative abundance of gram-positive bacteria than gram-negative bacteria. For the older, more shallow frazil ice samples, the gram-positive:gram-negative ratios were similar to those in the water (Figure 9). The percentage of the microbial population that was gram-negative increased, and the percentage that was gram-positive decreased with increasing depth in the frazil ice. This trend was accompanied by generally greater microbial biomass in deeper, more recently deposited frazil ice, that is probably also more porous. Gram-positive and gram-negative bacteria have different nutritional requirements and grow at different rates. Increases in microbial biomass with depth and differences in community types at different depths in frazil ice

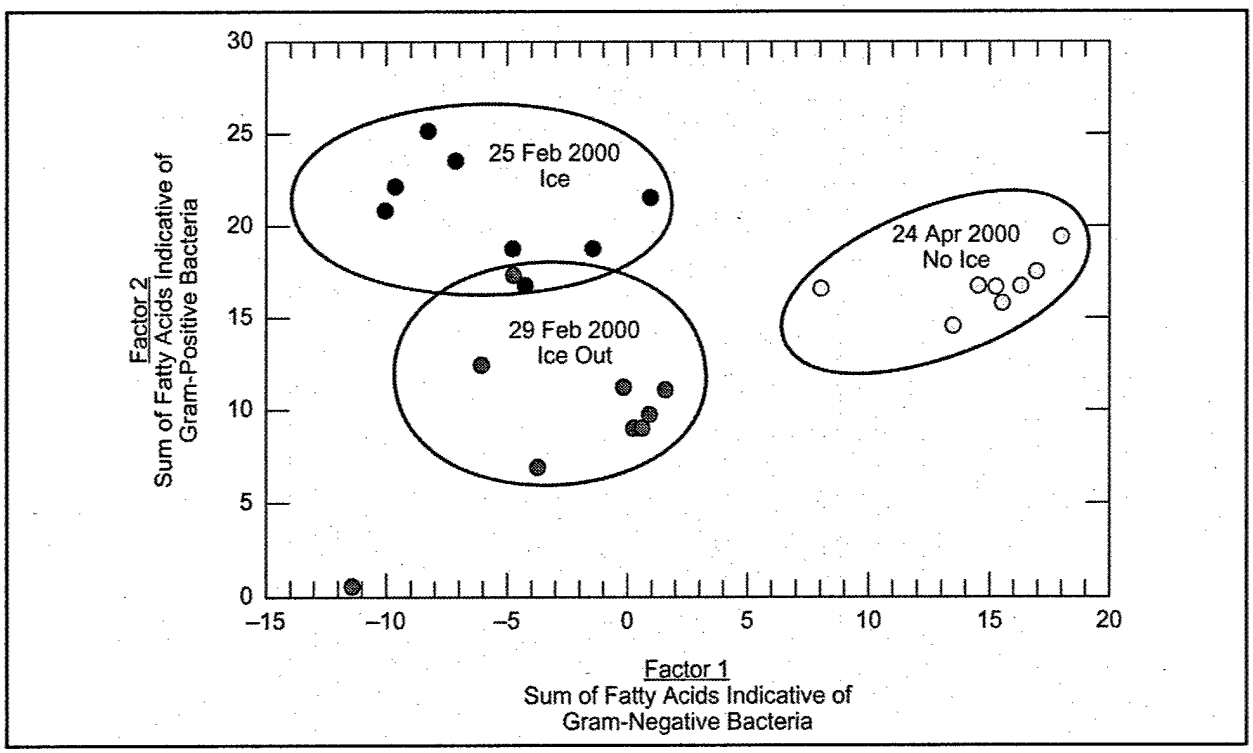

Figure 9. Results of the principal component analysis of fatty acid data obtained from culturable microorganisms collected from the LaPlatte River during an ice-covered period, immediately after ice-cover breakup, and well after ice-cover breakup, winter 1999-2000

may be due to greater availability of nutrients and carbon in the deeper, more recently deposited frazil ice. Greater porosity in the deeper frazil ice may allow for greater water and associated carbon flow and stimulate the gram-negative bacteria relative to the gram-positive bacteria. This environment could sustain higher metabolic activity similar to the greater activity that has been observed in the loose, outer fringes of biofilms compared to the inner regions of biofilms (Lock and John 1979, Paul and Duthie 1989). The higher microbial abundance in the lower portion of the frazil deposit could also result from the same processes that cause higher abundance in hyphoreic downwelling areas (Franken et al. 2001), a similar environment, and in sea ice (Palmisano and Sullivan 1983, Vincent 1988, Smith et al. 1989, Bowman et al. 1997). Following an examination of 135 bacterial species found in sea ice, Bowman et al. (1997) reported that 79 percent of the bacteria were gram negative.

Microbial Diversity. Microbial diversity can be examined using a number of statistical methods. In this study, a principal component analysis (PCA) of factors associated with gram-positive or gram-negative bacteria was used to compare fatty acid profiles among samples obtained during winter 1999-2000. Fatty acids were expressed as relative percentages within a given sample, and then transformed to obtain a normally distributed variable that would meet the assumptions of statistical testing. An arcsine square root transformation was used prior to comparison, as is commonly done with fatty acid data. Principal component analyses (PCA) and hierarchical cluster analyses (HCA) contained in the microbial identification library (MIDI, Inc. 1995) were used to compare fatty acid profiles of bacterial lawns grown from river water and ice samples for winter 2000-2001.

The results of the PCA analysis for LaPlatte River data obtained during winter 1999-2000 are shown in Figure 9, with ellipses added to highlight the groupings. The culturable microbial communities can clearly be separated into three distinct groups according to sampling date. These data clearly show that there are differences in the microbiology relative to sampling date; the data are not sufficiently sensitive to further define the differences and make further conclusons. Although the use of the 0.1X tryptic soy agar (TSA) media favors bacterial changes over fungal or algal changes, the data corroborate the respirometer data and support the concept that there are seasonal and ice-cover-related microbial community structure differences in the river. This finding is in agreement with the results of Grossman and Gleitz (1993), who found differences in community between the water and marine frazil ice in laboratory tests.

Particularly striking are the differences in microbial communities, as determined by the PCA, from samples taken on 25 and 29 February 2000, a period of four days that corresponded to dynamic ice cover breakup caused by sudden warm temperatures, precipitation, and rapid snowmelt. These data support the hypothesis that ice and subsequent ice breakup can affect riverine microbial community structure in fundamental ways. Both can also impact dissolved oxygen processes, although changes related to ice breakup are ephemeral, those associated with ice or frazil ice are not.

The results of the microbial studies during winter 1999-2000 indicated that fuller analyses should be undertaken during the second winter of data collection. As a result, microbial diversity in water samples collected during winter 2000-2001 was examined in two ways. First, a FAME PCA analysis similar to that performed in winter 1999-2000 was carried out on the TCA culturable microorganisms in samples that were obtained:

- At early ice cover formation, on 1 December 2000.
- In open water just following a breakup event on 19 December 2000.
- During the early ice-covered period on 3 January 2001.
- During the mid ice-covered period on 2 February 2001.
- In the late ice-covered period on 23 February 2001.
- In spring open water, 9 May 2001.

Following FAME analysis on whole-sample communities, individual isolates from the 2 February 2001 samples taken at two depths were grown and identified using FAME techniques and the FAME library.

The PCA results on whole-sample communities for winter 2000-2001 shown in Figure 10 cover the entire range of winter conditions, from early winter open water through spring open water. The results indicate that microbial communities in open water before ice cover formation and those just after ice cover formation are fairly closely related, but both are different than those found in springtime open water. Differences exist between communities found in mid- and late-ice-covered periods. The communities collected just after the breakup event are different from all of the other communities, again reflecting the exogenous input associated with the overbank flow event.

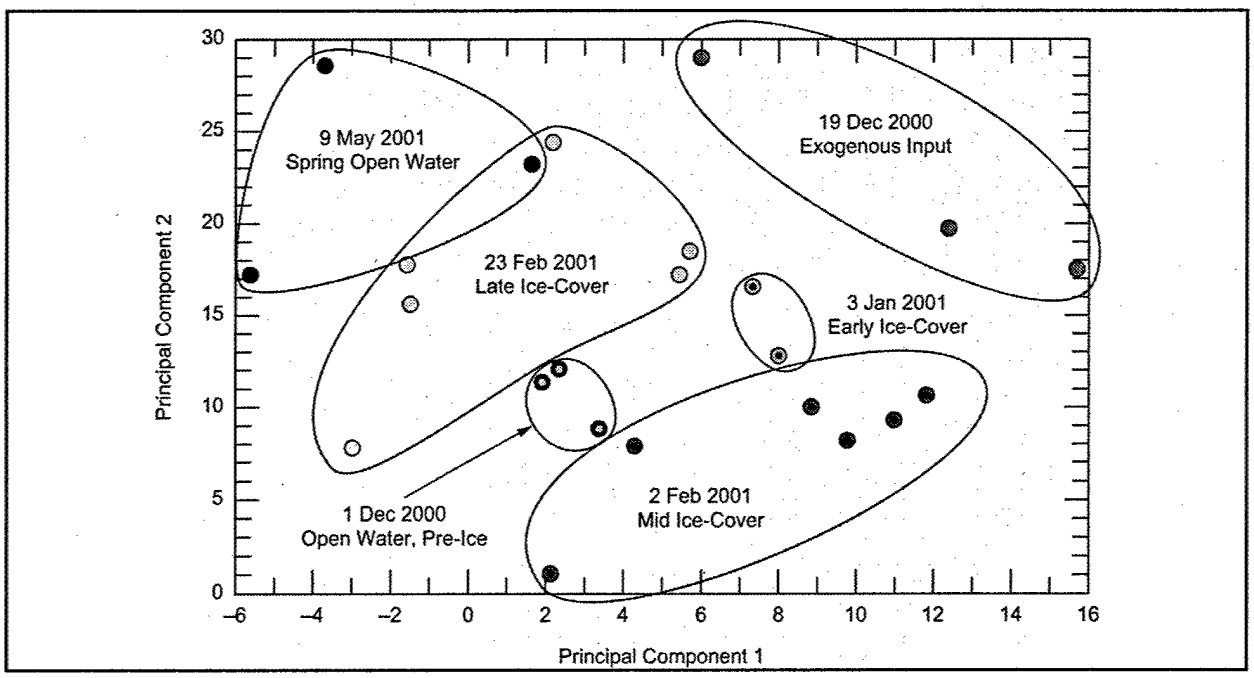

Figure 10. Results of the principal component analysis of fatty acid data obtained from culturable microorganisms collected from the LaPlatte River during winter 2000-2001

FAME analysis of individual isolates of the 2 February 2001 samples revealed that culturable bacteria did differ in community structure between the two depths sampled (Figure 11). Five genera were common to both shallow water just below the ice surface and deep water near the riverbed, and these genera occurred in approximately the same proportion. These were *Flavobacterium*, *Janthinobacterium*, *Pseudomonas*, *Kocuria*, and *Brevundimonas*. *Flavobacterium* are gram-negative facultative anaerobic bacteria, while *Janthinobacterium*, *Pseudomonas*, and *Brevundimonas* are gram-negative aerobes, and *Kocuria* are gram-positive aerobes. *Flavobacterium*, *Janthinobacterium*, and *Pseudomonas* are commonly found in soil and water. *Pseudomonas* species are capable of lignin degradation, an often important function in riverine systems (Atlas and Bartha 1998). *Flavobacterium* and *Pseudomonas* are very common psychrophilic organisms (Farrell and Rose 1967, Herbert 1986) and have been observed in sea ice algal-bacterial communities (Bowman et al. 1997).

Four unique genera occurred in shallow waters: *Psychrobacter*, *Acidovorans*, *Bordetella*, and *Cellulomonas*. The *Psychrobacter*, *Acidovorans*, and *Bordetella* genera are all gram-negative aerobes, while *Cellulomonas* is a non-spore-forming gram-positive facultative anaerobe that is found in soils (Holt et al. 1994). Three of the genera, *Psychrobacter*, *Bordetella*, and *Cellulomonas*, produce organic acids from glucose during metabolism, while the fourth, *Acidovorans*, feeds on organic acids. The *Psychrobacter* genera is the only true psychrotrophic bacteria identified (Holt et al. 1994).

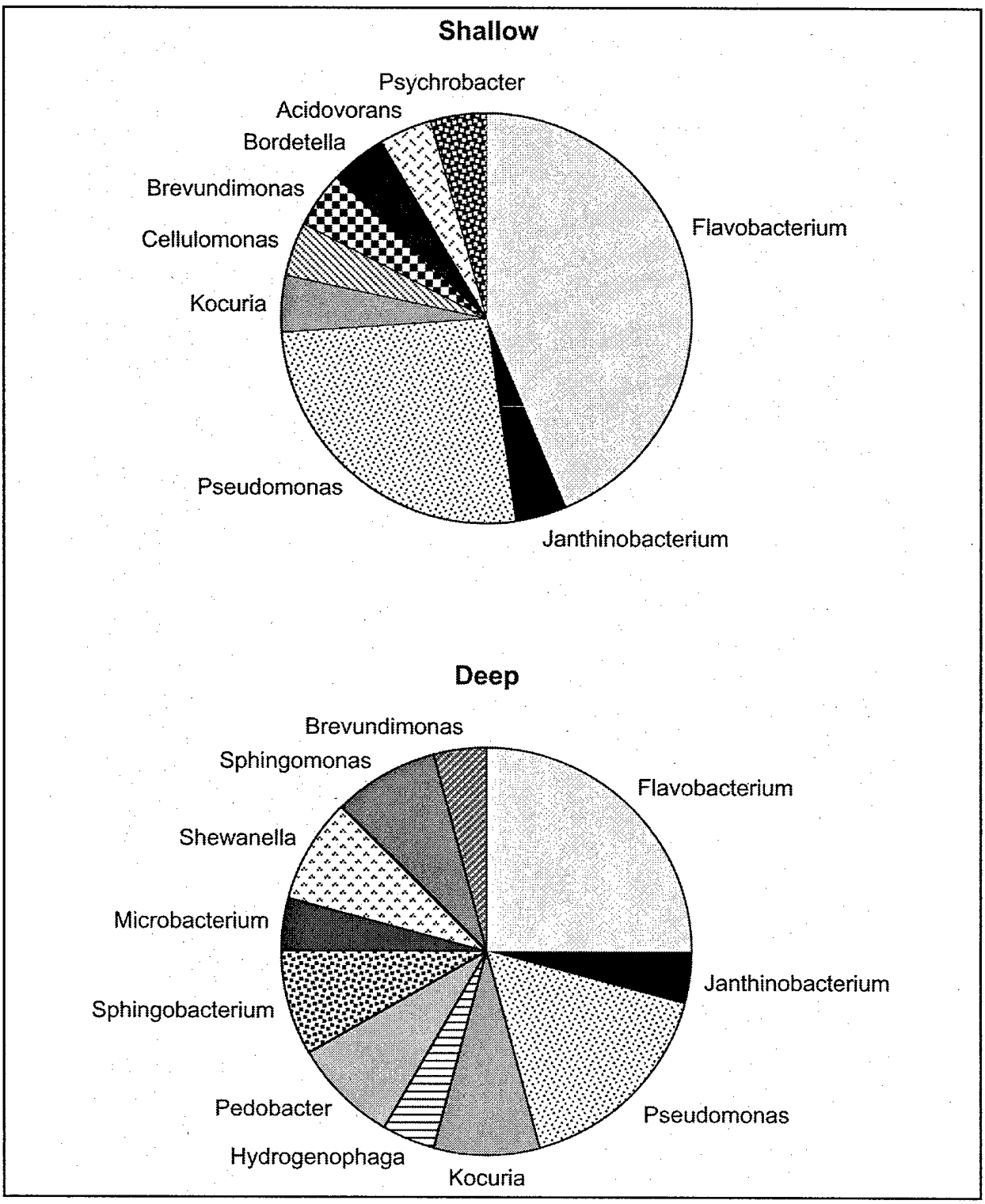

Figure 11. Percentage of different bacterial genera cultured from shallow water (just beneath the permanent ice layer) and deep water (just above the river bottom) samples from the LaPlatte River on 2 February 2001

Six unique genera were isolated from the deep waters, four of which can be classified as gram-negative facultative anaerobic bacteria and can grow under aerobic or anaerobic conditions: *Sphingomonas*, *Shewanella*, *Sphingobacterium*, and *Hydrogenophaga*. *Microbacterium* is a gram-positive aerobe. The sixth unique isolate in the deep water, *Pedobacter*, is a member of the *Cytophaga-Flavobacterium-Bacteriods-Sphingobacterium* group (Hiraishi et al. 2001) commonly found in fresh water and is a gram-negative aerobe. According to Bowman et al. (1997), the *Cytophaga-Flavobacterium-Bacteriods* group is known to occur in sea ice, as is *Shewanella*.

Microbial community differences were also observed at the Israel River site, where culturable microbial communities present were quantified using the modified FAME technique used for LaPlatte River samples. Using principal components analysis as a tool to group the FAME profiles for the culturable bacteria into two factors, a clear distinction between the water column community and the frazil ice communities was identifiable for all depths sampled (Figure 12).

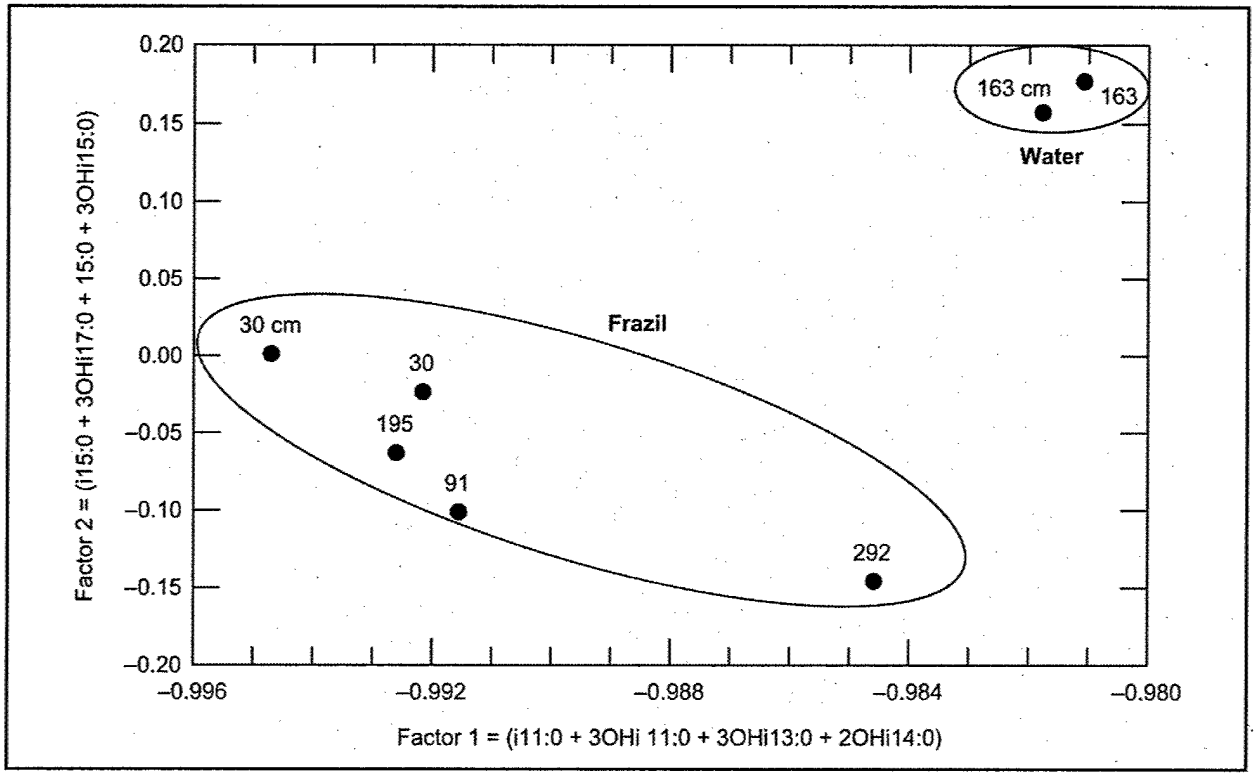

Figure 12. PCA of culturable microorganisms present in frazil ice and water samples collected at the Israel River, Lancaster, NH, on March 29, 2001

Estimates of Algal-Bacterial Assemblages. Although the preceding studies of microbial diversity concentrated on examining bacterial communities, the communities would be expected to be associated with algae, fungi, and protozoa in biofilm development within the ice based on reported observations in sea ice. For example, Grossman and Gleitz (1993) reported close metabolic coupling of algal-bacterial communities in the sea ice and in the open water during a series of experiments addressing microbial response to marine frazil ice formation. They also found 60 to 90 percent of bacteria attached to algae within the ice. Field measurements by Sullivan and Palmisano (1984) showed that only about 30 percent of bacteria were epiphytic (attached to algae) in

Antarctic sea ice. Similar percentages, about 37 percent, of epiphytic bacteria were reported by Smith et al. (1989) in Arctic sea ice. Another characteristic of algal-bacterial populations in sea ice is vertical stratification. As noted by Bowman et al. (1997), Palmisano and Sullivan (1983), Vincent (1988), and Smith et al. (1989), bacterial communities tend to be vertically stratified within sea ice, with high bacterial populations coinciding with high algal populations near the bottom, more loosely packed, portion of the ice.

The bacterial contribution to the algal-bacterial assemblages in terms of nutrient regeneration was addressed by Cota et al. (1991). They hypothesized that the bacteria increase seasonally as algal growth increases, yet make up a small percentage of the algal biomass. Their role is to recycle nitrogen and phosphorus through degradation, which in turn increases the supply of these limited nutrients to the algae. Based on studies of Antarctic sea ice communities, Vincent (1988) estimates that bacterial biomass was about 1 percent of algal biomass and that bacterial production is about 9 percent of algal primary production. Smith et al. (1989) found bacterial biomass up to 3 percent of algal biomass and estimated net bacterial production to be about 2.2 percent of net algal production in Arctic sea ice. They attributed about 40 percent of the bacterial production to epiphytic bacteria.

Algal abundance and productivity associated with the bacterial communities observed in the LaPlatte and Israel River biofilms may be estimated from relationships found in sea-ice algal-bacterial community structure. Bird and Kalff (1984) presented the following relationship for fresh and marine aquatic systems:

$$\log N = 8.87 \pm 0.06 + 0.78 \pm 0.05 \log C \quad (1)$$

where N is the bacterial cell concentration (numbers m^{-2}) and C is the chlorophyll *a* concentration (mg m^{-2}), a common indicator of algal cell numbers. Smith et al. (1989) reviewed data from Arctic and Antarctic sea ice and found a significant (r^2=0.57) relationship:

$$\log N = 10.76 \pm 0.05 + 0.27 \pm 0.03 \log C \quad (2)$$

Although they cautioned that it would be premature to compare this relationship to the one proposed by Bird and Kalff, it is more conservative and thus is preferred for use in estimating algal concentrations from bacterial abundance in river ice.

Bacterial abundance of frazil ice samples obtained for the LaPlatte River and the Israel River are summarized in Table 3. The chlorophyll *a* concentration of the frazil is also estimated, assuming the relationship shown in Equation 2 is valid for freshwater river ice. APHA (1995) suggests that the algal biomass can be obtained by multiplying the chlorophyll *a* content by a factor of 0.67, resulting in the estimated algal biomass values shown in Table 3. The average chlorophyll *a* concentration for the LaPlatte River and for the deepest frazil sample taken from the Israel River is about 2 X 10^{-4} mg m^{-3}. As a comparison, Haack and McFeters (1982) provide chlorophyll *a* and bacterial data for benthic algal-bacterial mats in the outlet stream of an alpine cirque lake in Montana. The ice cover had recently broken up on the lake, water temperature was about 5 °C, and bacterial concentration was 4.35 X 10^{12} per m^2. Using Equation 2, the chlorophyll *a* concentration would be about 9 X 10^6 m^{-2}. This compares favorably to their reported value of about 8.5 X 10^6 m^{-2}, so there is some

confidence that the relationship developed for marine sea ice could be used in a cold freshwater system.

Table 3
Summary of Bacterial Cell Concentrations in LaPlatte and Israel River Frazil Deposits with Estimated Chlorophyll *a* and Algal Biomass Concentrations

River	Sample Date	Bacterial Cell Concentration (CFU mL^{-1})	Bacterial Cell Concentration (Numbers m^{-2})	Chlorophyll *a* Concentration (mg m^{-2})	Algal Biomass (mg m^{-3})
LaPlatte River	1/3/01	6.6×10^{4}	6.6×10^{8}	4.5×10^{-4}	0.16
Israel River, 30 cm	3/29/01	3.6×10^{4}	3.6×10^{8}	4.8×10^{-5}	1.67×10^{-2}
Israel River, 60 cm	3/29/01	2.2×10^{4}	2.2×10^{8}	7.7×10^{-6}	2.7×10^{-3}
Israel River, 162 cm	3/29/01	5.3×10^{4}	5.3×10^{8}	2.0×10^{-4}	7.0×10^{-2}
Israel River, 258 cm	3/29/01	6.7×10^{4}	6.7×10^{8}	4.8×10^{-4}	0.17

Chlorophyll *a* is also used as an indicator of trophic status. Carlson (1977) developed a widely used trophic status index (TSI) for lakes based on surface chlorophyll *a* concentration:

$$TSI = 10\left(6 - \frac{2.04 - 0.68\ln(Chl)}{\ln 2}\right) \tag{3}$$

where *Chl* is the surface chlorophyll a concentration in mg m^{-3}. Unfortunately, his relationship was developed from a regression that specifically excluded wintertime values, and thus is suitable for use here only for qualitative or comparison purposes. Using the average winter chlorophyll *a* concentration estimated for the LaPlatte River and for the deepest frazil sample taken from the Israel River, the Carlson TSI would be 0, representing an ultraoligotrophic state.

Using the Organization for Economic Co-operation and Development (OECD 1982) fixed boundary system classification, the LaPlatte and Israel Rivers would be classified as oligotrophic to ultraoligotrophic in wintertime. This system classifies ultraoligotrophy as occurring when mean chlorophyll *a* concentration is less than 1.0 mg m^{-3} and peak chlorophyll *a* concentration is less than 2.5 mg m^{-3}. The classification results using either system are not unreasonable given the characterization of the riverine environment as nutrient-poor compared to the lacustrine environment for which these TSIs were developed. The characterization of low productivity using these estimates is consistent with the small amplitude of diurnal DO cycling observed on the LaPlatte River (White 2002, White et al. 2001).

CONCLUSIONS AND RECOMMENDATIONS: This study used field and laboratory observations of microbial abundance and diversity to examine microbial community structure in ice-covered rivers located in northern New England. Microbial abundance measured in the LaPlatte River over a four-month period during winter 2000-2001 showed a general, though not statistically significant, decline in CFU beginning at the time of intermittent ice-cover formation through the late ice-cover period. Biomass increased after ice-cover breakup. Measured microbial abundance was similar to that noted in ice-covered rivers by other researchers. An early winter ice-cover breakup event that was accompanied by precipitation, snowmelt, and overbank flow resulted in an order of magnitude increase in CFU that soon decreased to original levels. Fatty acid analysis showed that

the community structure was different under late-ice-cover conditions, just after ice-cover breakup, and in springtime open water.

Bacterial abundance within a deep frazil deposit on the Israel River tended to increase with depth. Abundance within the deposit was greater than in the water column. Other researchers in the marine environment have noted similar results. Based on estimates of algal biomass made from bacterial abundance, both rivers can be classified in the oligotrophic to ultraoligotrophic range, which agrees well with small-amplitude diurnal cycling of DO on the Laplatte River.

Further research is necessary to characterize biofilm development in river ice covers and at the ice-water interface, particularly the algal component and algal-bacterial interactions. Studies involving culturing of bacteria, as was the case in this study, run the risk of missing significant non-culturable bacteria. The use of phospholipid ester-linked fatty acid analysis of samples extracted directly from water may avoid problems with culturing microorganisms and provide enumeration and identification with less uncertainty. The use of recent identification techniques utilizing DNA, RNA, or genetic probes could also provide more complete information on the nature of the biofilm community and the changes that it undergoes throughout the winter season.

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